ÖGH Herpetozoa
Austrian Herpetological Society

Herpetozoa 37: 111–121 (2024)
DOI 10.3897/herpetozoa.37.e120524

Range extension and expanded description of *Micryletta hekouensis* Liu, Hou, Mo & Rao, 2021 (Amphibia, Anura, Microhylidae), with comments on *Micryletta* of Northern Vietnam

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Academic editor: Günter Gollmann ♦ **Received** 8 February 2024 ♦ **Accepted** 3 April 2024 ♦ **Published** 23 April 2024

Abstract

The Paddy frog species *Micryletta hekouensis* was described based on only two specimens from Nanxi Village, Nanxi Town, Hekou County, Honghe Prefecture, Yunnan Province, China. Herein, we report on new findings and a range extension of this species based on a re-examination of preserved specimens deposited in Duy Tan University (DTU) and Zoological Museum of Lomonosov Moscow State University (ZMMU) collected from Vietnam. All new specimens were previously identified as *Micryletta* cf. *inornata* or *M.* cf. *steinegeri*. Molecular analyses based on mitochondrial DNA supported the morphological findings. The newly identified specimens have a pairwise divergence of only 0.7% from those of the type series of *M. hekouensis*, based on the 16s rRNA mtDNA gene. Based on the new information, we confirm the presence of *M. hekouensis* in Vietnam and update the diagnostic characters of this species and its distribution. We suggest the species should be considered as Near Threatened (NT) following the IUCN's Red List categories. Further studies reassessing the populations of the *Micryletta inornata* complex in the Indochinese Region (including Vietnam, Laos, Cambodia, and Thailand) are required.

Key Words

Cat Ba National Park, Cuc Phuong National Park, *Micryletta inornata*, *Micryletta steinegeri*, morphology, mtDNA, new record, phylogeny, redescription

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Introduction

The Paddy frogs of the genus *Micryletta* Dubois, 1987 are a little-known group of microhylids, with 13 nominal species currently recognized, namely: *M. aishani* Das, Garg, Hamidy, Smith & Biju, 2019; *M. dissimulans* Suwannapoom, Nguyen, Pawangkhanant, Gorin, Chomdej, Che & Poyarkov, 2020; *M. erythropoda* (Tarkhnishvili, 1994); *M. hekouensis* Liu, Hou, Mo & Rao, 2021; *M. immaculata* Yang & Poyarkov, 2021; *M. inornata* (Boulenger, 1890); *M. lineata* (Taylor, 1962); *M. melanops* Poyarkov, Nguyen, Yang & Gorin, 2021; *M. menglienica* (Yang & Su, 1980); *M. nigromaculata* Poyarkov, Nguyen, Duong, Gorin & Yang, 2018; *M. steinegeri* (Boulenger, 1909); *M. subaraji* Sankar, Law, Law, Shivaram, Abraham & Chan, 2022; and *M. sumatrana* Munir, Hamidy, Matsui, Kusrini & Nishikawa, 2020 (Sankar et al. 2022; Frost 2024). However, the small body size, elusive habits, as well as the remarkable morphological similarity of some of the *Micryletta* species complicates taxonomic studies of this group. Therefore, the taxonomic diversity of the genus *Micryletta* is not yet fully realized and requires additional studies. Presently, four nominal *Micryletta* species are recorded from Vietnam, including *Micryletta erythropoda*, *M. melanops*, *M. menglienica*, and *M. nigromaculata*. Two other species, namely *Micryletta inornata* (restricted to Sumatra Island and Southern Myanmar) and *M. steinegeri* (restricted to the Taiwan Island of China), have been reported from Vietnam in previous works (Nguyen et al. 2009; Poyarkov et al. 2018, 2021b), but recent phylogenetic studies suggest that these records were likely based on misidentifications with either *Micryletta* cf. *immaculata* or *M. menglienica* or *M. hekouensis* (Miller et al. 2021; Poyarkov et al. 2021b; Sankar et al. 2022; see Discussion).

The Hekou Paddy Frog, *Micryletta hekouensis* was described based on one male and one female specimen, both originating from Nanxi Village, Nanxi Town, Hekou County, Honghe Prefecture, Yunnan Province, China (Liu et al. 2021a). To date, this species was known only from its type locality in China. The species is characterized by: comparatively small body size (SVL 20.5 mm in male, 20.8 mm in female); areas above the canthus rostralis, upper eyelids, areas posterior to eyelids, and dorsum of upper arms golden, other parts of the dorsum are almost solid black or yellowish grey with brownish black stripes; lateral sides of head and body black or yellowish grey, a white stripe from lower front of eye along upper lip back to anterior forelimb insertion; ventral side of body and limbs is pink brown, chin region in adult males brownish black, small and irregular white marbling patterns on chest and lateral belly; supratympanic fold indistinct; outer metatarsal tubercle absent; webbing between toes absent; tibiotarsal articulation adpressed limb reaching level of front of eye (Liu et al. 2021a).

The recent molecular results of Miller et al. (2021) and Sankar et al. (2022) revealed that populations previously identified as *Micryletta* cf. *inornata* or *M.* cf. *steinegeri* in the Ninh Binh and Hai Phong in Northern Vietnam reported by Poyarkov et al. (2018) were nested within the same lineage as the holotype and paratype of *Micryletta hekouensis*, implying that this species has a more extensive distribution than currently recognized. To address this question, we re-examined specimens previously identified as *Micryletta* cf. *inornata* or *M.* cf. *steinegeri* from Ninh Binh and Hai Phong Provinces deposited in the zoological collections of the Duy Tan University (**DTU**, Vietnam) and Zoological Museum of Lomonosov Moscow State University (**ZMMU**, Russia), respectively. Our results confirm that the specimens from Ninh Binh Province as well as Hai Phong Province belong to *Micryletta hekouensis*. We herein formally confirm the occurrence of *Micryletta hekouensis* in Vietnam, update the distribution of this species, provide additional data on its natural history and revise its diagnostic characters.

Materials and methods

Material examined

We examined twelve specimens that were previously registered as *M.* cf. *inornata* or *M.* cf. *steinegeri* from Cuc Phuong National Park (hereafter **NP**), Ninh Binh Province and Cat Ba NP, Hai Phong Province, Northern Vietnam (Poyarkov et al. 2018) (see Table 1). Morphological comparisons were based on literature data from: Boulenger (1890); Das et al. (2019); Liu et al. (2021a,b); Miller et al. (2021); Munir et al. (2020); Poyarkov et al. (2018, 2021b); Sankar et al. (2022); Suwannapoom et al. (2020); Tarkhnishvili (1994); Taylor (1962); Yang and Poyarkov (2021) (see Suppl. material 1).

Morphological analyses

Measurements were taken using a digital caliper under a light dissecting microscope to the nearest 0.01 mm, subsequently rounded to 0.1 mm. The morphometrics of adults and character terminology followed Nguyen et al. (2020), including **SVL**: snout-vent length, **HL**: head length (from the back of mandible to tip of snout), **HW**: maximum head width (across angles of jaws), **SL**: snout length (from anterior corner of eye to tip of snout), **NSD**: distance from nostril to the tip of snout, **END**: distance from anterior corner of eye to the nostril, **IND**: internarial distance, **IOD**: interorbital distance, **ED**: eye diameter, **UEW**: maximum width of upper eyelid, **TD**: tympanum diameter, **TYED**: distance from anterior margin of tympanum to posterior corner of the eye, **FLL**: forearm length (from axilla to elbow), **HAL**: hand length (from elbow to the tip of third finger), **FL1–4**:

finger length I–IV, **OPTL**: outer palmar tubercle length, **IPTL**: inner palmar tubercle length, **NPL**: nuptial pad length, **FeL**: femur length (from vent to knee), **TbL**: tibia length (from knee to tarsus), **TbW**: maximum tibia width, **FoL**: foot length (from tarsus to the tip of fourth toe), **TL 1–5**: toe length I–V, **IMTL**: inner metatarsal tubercle length, **FD3D**: maximal diameter of disk of finger III, and **TD4D**: maximal diameter of disk toe IV. Terminology for describing eye colouration in living individuals followed Glaw and Vences (1997); subarticular tubercle formulas and webbing formula followed those of Savage (1975). All measurements were taken on the right side of the examined specimen. Sex was determined by gonadal inspection following dissection.

Table 1. Sequences (16S rRNA) used in molecular analyses of this study.

No.	Previously taxon	Proposed taxon	Voucher	Locality	Accession	Reference
1	*M. aishani*	*M. aishani*	SDBDU 3920	India: Assam, Cachar, Subhong	MK889218	Das et al. (2019)
2	*M. aishani*	*M. aishani*	CAS 231526	Myanmar: Kachin, Indawgyi WS	MW035599	Miller et al. (2021)
3	*M. dissimulans*	*M. dissimulans*	AUP 01690	Thailand: Songkla, Saba Yoi	MT573414	Suwannapoon et al. (2020)
4	*M. dissimulans*	*M. dissimulans*	AUP 01691	Thailand: Songkla, Saba Yoi	MT573415	Suwannapoon et al. (2020)
5	*M. dissimulans*	*M. dissimulans*	AUP 01696	Thailand: Songkla, Saba Yoi	MT573416	Suwannapoon et al. (2020)
6	*M. dissimulans*	*M. dissimulans*	AUP 01698	Thailand: Songkla, Saba Yoi	MT573413	Suwannapoon et al. (2020)
7	*M. erythropoda*	*M. erythropoda*	ZMMU A4721-1533	Vietnam: Dong Nai, Ma Da N.R.	MH756146	Poyarkov et al. (2018)
8	*M. erythropoda*	*M. erythropoda*	ZMMU A4721-1542	Vietnam: Dong Nai, Ma Da N.R.	MH756147	Poyarkov et al. (2018)
9	*M. hekouensis*	*M. hekouensis*	KIZ 20210510	China: Yunnan, Honghe, Hekou	MZ536627	Liu et al. (2021a)
10	*M. hekouensis*	*M. hekouensis*	KIZ 20210511	China: Yunnan, Honghe, Hekou	MZ536628	Liu et al. (2021a)
11	*M.* cf. *inornata*	*M. hekouensis*	DTU 310	Vietnam: Ninh Binh, Cuc Phuong N.P.	PP264232	This study
12	*M.* cf. *inornata*	*M. hekouensis*	DTU 311	Vietnam: Ninh Binh, Cuc Phuong N.P.	PP264231	This study
13	*M.* cf. *inornata*	*M. hekouensis*	DTU 312	Vietnam: Ninh Binh, Cuc Phuong N.P.	PP264230	This study
14	*M.* cf. *inornata*	*M. hekouensis*	ZMMU NAP-3352-1	Vietnam: Hai Phong, Cat Ba N.P.	MH879843	Poyarkov et al. (2018)
15	*M.* cf. *inornata*	*M. hekouensis*	ZMMU NAP-3352-2	Vietnam: Hai Phong, Cat Ba N.P.	MH879844	Poyarkov et al. (2018)
16	*M.* cf. *inornata*	*M. hekouensis*	ZMMU NAP-3580	Vietnam: Hai Phong, Cat Ba N.P.	MH879845	Poyarkov et al. (2018)
17	*M. immaculata*	*M. immaculata*	KFBG 14270	China: Hainan, Exian	MW376736	Yang and Poyarkov (2021)
18	*M. immaculata*	*M. immaculata*	KFBG 14271	China: Hainan, Exian	MW376737	Yang and Poyarkov (2021)
19	*M. inornata*	*M. immaculata*	FMNH 255121	Laos: Khammouan, Boualapha	KC179997	de Sa et al. (2012)
20	*M. inornata*	*M. immaculata*	TZ9892	Vietnam: Ha Tinh, Ke Go	AF285206	Ziegler (2002)
21	*M. inornata*	*M. inornata*	MZB 23949	Indonesia: Sumatra, Deli Serdang	LC208135	Alhadi et al. (2019)
22	*M. inornata*	*M. inornata*	MZB 23947	Indonesia: Sumatra, Deli Serdang	LC208136	Alhadi et al. (2019)
23	*M. inornata*	*M. inornata*	MZB 23948	Indonesia: Sumatra, Deli Serdang	LC208137	Alhadi et al. (2019)
24	*M. inornata*	*M. inornata*	MZB 27242	Indonesia: Sumatra, Aceh	LC208138	Alhadi et al. (2019)
25	*M. inornata*	*M. inornata*	USNM 587625	Myanmar: Tanintharyi	MT609033	Miller et al. (2021)
26	*M. inornata*	*M. inornata*	USNM 587901	Myanmar: Tanintharyi	MT609034	Miller et al. (2021)
27	*M. inornata*	*M. lineata*	KUHE 23858	Thailand: Ranong	AB634695	Matsui et al. (2011)
28	*M. inornata*	*M. lineata*	CAS 247206	Myanmar: Tanintharyi, Kawthaung	KM509167	Peloso et al. (2015)
29	*M. melanops*	*M. melanops*	ZMMU NAP-00449	Vietnam: Lam Dong, Biduop-Nui Ba N.P.	MZ474684	Poyarkov et al. (2021b)
30	*M. melanops*	*M. melanops*	ZMMU NAP-01381	Vietnam: Lam Dong, Biduop-Nui Ba N.P.	MZ474685	Poyarkov et al. (2021b)
31	*M. menglienica*	*M. menglienica*	KIZ 20210708	China: Yunnan, Pu'er, Menglian	OK335183	Liu et al. (2021b)
32	*M. menglienica*	*M. menglienica*	KIZ 20210709	China: Yunnan, Pu'er, Menglian	OK335184	Liu et al. (2021b)
33	*M. menglienica*	*M. menglienica*	KFBGF 14653	China: Yunnan, Xishuangbanna, Mengla	OR053962	Yeung et al. (2023)
34	*M. inornata*	*M. menglienica*	KUHE 20497	Thailand: Phrae, Mae Yom	AB598341	Matsui et al. (2011)
35	*M. inornata*	*M. menglienica*	K 3068	Thailand: Chiang Mai, Doi Chiang Dao	KR827953	Grosjean et al. (2015)
36	*M. inornata*	*M. menglienica*	K 3246	Laos: Luangprabang, Ban Sop Chuna	KC180027	Grosjean et al. (2015)
37	*M. nigromaculata*	*M. nigromaculata*	ZMMU A5947	Vietnam: Hai Phong, Cat Ba N.P.	MH756148	Poyarkov et al. (2018)
38	*M. nigromaculata*	*M. nigromaculata*	ZMMU A5937	Vietnam: Hai Phong, Cat Ba N.P.	MH756149	Poyarkov et al. (2018)
39	*M. nigromaculata*	*M. nigromaculata*	ZMMU A5946	Vietnam: Hai Phong, Cat Ba N.P.	MH756151	Poyarkov et al. (2018)
40	*M. nigromaculata*	*M. nigromaculata*	DTU 301	Vietnam: Ninh Binh, Cuc Phuong N.P.	MH756154	Poyarkov et al. (2018)
41	*M. steinegeri*	*M. steinegeri*	KUHE 35937	China: Taiwan, Yunlin	AB634696	Matsui et al. (2011)
42	*M. steinegeri*	*M. steinegeri*	ZMMU A5336-1	China: Taiwan, Kaohsiung	MW376732	Poyarkov et al. (2018)
43	*M. steinegeri*	*M. steinegeri*	ZMMU A5336-2	China: Taiwan, Kaohsiung	MW376733	Poyarkov et al. (2018)
44	*M. steinegeri*	*M. steinegeri*	ZMMU A5336-3	China: Taiwan, Kaohsiung	MW376734	Poyarkov et al. (2018)
45	*M. subaraji*	*M. subaraji*	ZRC1.13370	Singapore: Kranji Marshes	ON026065	Sankar et al. (2022)
46	*M. subaraji*	*M. subaraji*	ZRC 1.13369	Singapore: Kranji Marshes	ON026064	Sankar et al. (2022)
47	*M. subaraji*	*M. subaraji*	ZRC 1.13389	Singapore: Kranji Marshes	ON026066	Sankar et al. (2022)
48	*M. subaraji*	*M. subaraji*	ZRC 1.13323	Singapore: Kranji Marshes	ON026063	Sankar et al. (2022)
49	*M. sumatrana*	*M. sumatrana*	MZB 30594	Indonesia: Sumatra Selatan	MN727065	Munir et al. (2020)
	Our group					
50	*Kaloula pulchra*	*Kaloula pulchra*	NMNS 3208	China	KC822614	Blackburn et al. (2013)
51	*Kaloula pulchra*	*Mysticellus franki*	ZSI/WGRC/V/A/967	India: Kerala, Wayand	MK285340	Garg and Biju (2019)
52	*Kaloula pulchra*	*Uperodon systoma*	SDBDU 2005.4723	India: Tamil Nadu: Kunnapattu	MG557949	Garg and Biju (2019)

Molecular phylogeny

We synthesized previously published sequences of the *Micryletta steinegeri* members from GenBank to estimate the phylogenetic relationships of the genus *Micryletta* and genetically identity samples referable to *M. hekouensis*. We focused on sequences for the mitochondrial 16S rRNA gene as it is phylogenetically informative for most Paddy frogs and has the largest availability of any gene for *Micryletta*. As the sequences of the three specimens (DTU 310-12) provided by Poyarkov et al. (2018) are too short to obtain a stable phylogenetic position, we obtained longer sequences of these three specimens and uploaded them to GenBank. We aligned the 16S sequences of 13 species of *Micryletta*; we used the sequences of *Kaloula pulchra* Gray, 1831; *Mysticellus franki* Garg & Biju, 2019; and *Uperodon systoma* (Schneider, 1799) to root the tree (GenBank accession numbers, voucher specimens, locality, and source information are summarized in Table 1).

Sequences were aligned using MUSCLE (Edgar 2004) integrated in MEGA 11 (Tamura et al. 2021) with default parameters. Genetic divergences (uncorrected p-distance) were calculated in MEGA 11. The best substitution models were selected using the Akaike Information Criterion (AIC) in ModelFinder (Kalyaanamoorthy et al. 2017). Maximum likelihood phylogenetic analysis was performed in IQ-TREE 1.6.12 (Nguyen et al. 2015) based on the TIM2+F+I+G4 model, and nodal support was estimated by 1,000 ultrafast bootstrap (UFB) replicates. Nodes with UFB values of 95 and above were considered significantly supported (Minh et al. 2013). Bayesian Inference was performed in MrBayes 3.2.7 (Ronquist et al. 2012) based on the GTR+F+I+G4 model. Two runs were performed simultaneously with four Markov chains starting from a random tree. The chains were run for 5,000,000 generations and sampled every 100 generations. The first 25% of the sampled trees were discarded as burn-in and then the remaining trees were used to estimate Bayesian posterior probabilities (BPPs). Nodes were considered well-supported if they had BPPs of 0.95 or higher (Huelsenbeck et al. 2001; Wilcox et al. 2002).

Results

The ML and BI analyses of 16S rRNA sequences recovered trees with similar topologies (Fig. 1). With respect to the position of *M. hekouensis*, our phylogenetic results largely conform to those of Poyarkov et al. (2018), Miller et al. (2021), Liu et al. (2021a) and Sankar et al. (2022). The reconstructed phylogenetic relationship indicates that four species, namely *M. steinegeri*, *M. menglienica*, *M. immaculata*, and *M. hekouensis* are nested within a single monophyletic clade *M. steinegeri* complex (Fig. 1).

The uncorrected *p*-distances of the 16s gene fragment among examined members of the *M. steinegeri* species groups are presented in Table 2. Six sequences of *Micryletta* spp (PP264232 [DTU 310], PP264231 [DTU 311], PP264230 [DTU 312], respectively, reported from Cuc Phuong NP., Ninh Binh Province as well as MH879843 [ZMMU NAP-3352-1], MH879844 [ZMMU NAP-33521–2], MH879845 [ZMMU NAP-3580], respectively, reported from Cat Ba NP., Hai Phong Province, Vietnam clustered with the type series of *M. hekouensis* from Nanxi Town, Hekou County, Honghe Prefecture, Yunnan Province, China, and were only 0.7% divergent from the type series of *M. hekouensis*.

Furthermore, we also examined the morphology of other *Micryletta* specimens previously registered as *Micryletta* cf. *inornata* or *M.* cf. *steinegeri* from Ninh Binh and Hai Phong including five specimens DTU 310–312 as well as ZMMU NAP-3580, ZMMU NAP-3580 and found morphological similarities with *M. hekouensis*. These results support our hypothesis that previous records of *M. inornata* (restricted to Sumatra Island in Indonesia, southern Myanmar) and *M. steinegeri* (restricted to Taiwan Island in China) in Northern Vietnam (Ninh Binh and Hai Phong province), should be referred to as *M. hekouensis*. Therefore, we extend the distribution of *Micryletta hekouensis* to Vietnam and provide an expanded diagnosis and description.

Table 2. Uncorrected p-distances (%) of 16S rRNA sequences between the species of *Micryletta*.

Species	1	2	3	4	5	6	7	8	9	10	11	12	13
1 *Micryletta aishani*													
2 *Micryletta dissimulans*	4.4												
3 *Micryletta erythropoda*	4.8	7.4											
4 *Micryletta hekouensis* (China)	3.6	5.0	6.5										
5 *Micryletta hekouensis* (Vietnam)	3.0	4.7	6.3	0.7									
6 *Micryletta immaculata*	4.3	6.3	6.8	3.9	3.5								
7 *Micryletta inornata*	5.0	5.9	7.6	5.5	5.1	7.0							
8 *Micryletta lineata*	3.4	6.0	3.1	4.6	4.4	5.4	6.3						
9 *Micryletta melanops*	3.0	4.4	7.1	5.9	5.6	5.9	5.6	5.5					
10 *Micryletta menglienica*	3.6	5.4	6.4	3.2	2.8	2.6	6.6	4.8	5.4				
11 *Micryletta nigromaculata*	4.7	5.2	8.3	8.1	7.6	7.5	6.6	7.2	6.8	7.1			
12 *Micryletta steinegeri*	3.6	4.8	6.7	3.1	2.8	3.6	5.5	5.1	6.5	2.8	7.2		
13 *Micryletta subaraji*	4.6	5.2	7.5	6.6	6.3	6.9	2.9	6.4	7.1	6.5	7.9	7.1	
14 *Micryletta sumatrana*	5.9	5.1	9.1	6.7	6.3	7.8	8.1	7.4	6.2	7.1	5.5	6.0	8.9

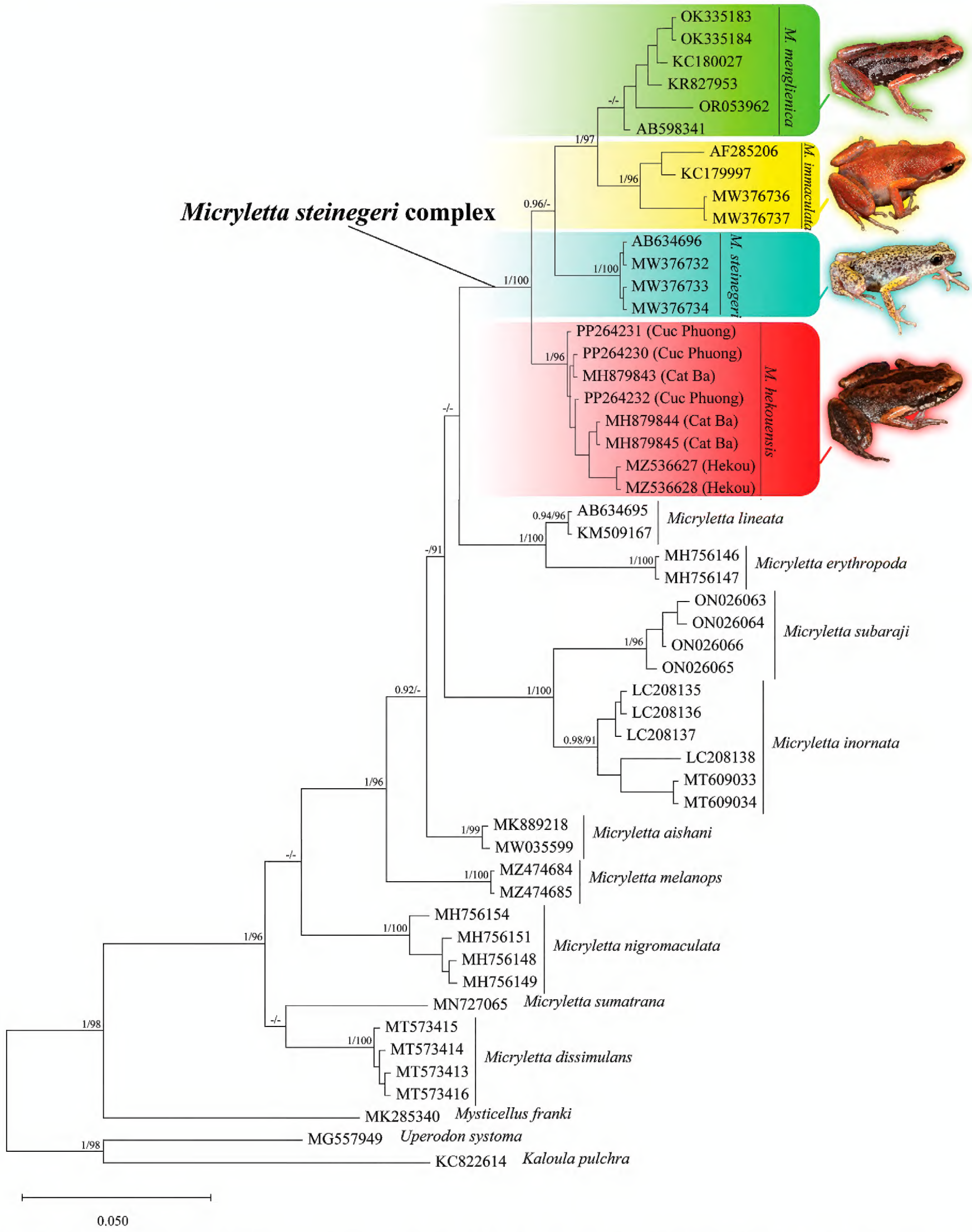

Figure 1. Maximum Likelihood (ML) tree of *Micryletta* derived from the analysis of 16s mitochondrial DNA gene sequences. For voucher specimen information and GenBank accession numbers see Table 1. Numbers at tree nodes correspond to ML UFBS/BI PP support values, respectively. Photos by J.B Zhao, J.H. Yang, C.W. You, and N.A. Poyarkov.

Taxonomic account

Micryletta hekouensis

Table 3, Fig. 2

Micryletta cf. *inornata* – Poyarkov et al. (2018: 1–27, in part); Miller et al. (2021: 248, in part).

Micryletta cf. *steinegeri* – Poyarkov et al. (2021a: 42–43, in part).

Micryletta hekouensis – Liu, Hou, Mo & Rao (2021: 133).

Micryletta hekouensis – Sankar et al. (2022: 462).

Holotype. KIZ 20210510, adult male collected by Shuo Liu on 15 May 2021. **Type locality:** Nanxi Village, Nanxi Town, Hekou County, Honghe Prefecture, Yunnan Province, China (22°38'17"N, 103°59'8"E; elevation 350 m a.s.l.).

Suggested name in Vietnamese. Nhái bầu Hà khẩu.

Specimens examined (n = 12). Two adult males DTU 310, 316 and four adult females DTU 309, 311–312, 317 from Cuc Phuong NP, Ninh Binh Province, Vietnam (ca. 20.2594°N, 105.6928°E; elevation of 160–215 m a.s.l) collected by Tan Van Nguyen on 3 June 2018. Three adult males ZMMU NAP-2176, ZMMU NAP-3580, ZMMU NAP-5572 and three adult females ZMMU NAP-3352, ZMMU NAP-3574, ZMMU NAP-5574 from Cat Ba NP, Hai Phong Province, Vietnam (ca. 20.8123°N, 106.9988°E; elevation of 90 m a.s.l.) collected by Nikolay A. Poyarkov in October 2013.

Variation based on examined specimens from Vietnam (n = 12). According to the original description of Liu et al. (2021a) the species *M. hekouensis* is a small-sized species of *Micryletta* with SVL 20.5 mm in adult male (Holotype, KIZ 20210510) and SVL 20.8 mm in adult female (Paratype, KIZ 20210511). However, the specimens from Vietnam exhibit generally larger body sizes: SVL 20.0–24.1 mm in males (n = 5), 25.4–29.5 mm in females (n = 7). Therefore, we propose that the paratype specimen should be considered a subadut female, and that *M. hekouensis* in fact represents a medium-sized species within its genus. Moreover, the TbL/SVL ratio in female specimens from Vietnam (0.43–0.55) was also slightly larger than in the paratype female from China (0.40). Furthermore, Liu et al. (2021a) reported this species to have a small rounded and distinct tympanum; however, the specimens from Vietnam had the tympanum hidden. It is not clear if the observed morphological differences between the type series of *M. hekouensis* from China and the series from Vietnam represent the actual intraspecific variation, or might result from the different state of specimen preservation, the reproductive condition of the specimens, or the observer effect. However, though we acknowledge the limitations of mtDNA markers for species delimitation in amphibians (e.g., Velo-Antón et al. 2023), the overall similarity of the Chinese and Vietnamese specimens of *M. hekouensis* in external morphological traits and coloration strongly suggest that they belong to the same species.

Additionally, the Vietnamese specimens examined were characterized by: habitus relatively slender; head small and triangular, width approximately equal to length (HW/HL 0.93–1.11 in males, 0.97–1.22 in females); snout abruptly rounded in dorsal view and slightly acuminate in profile, projecting beyond margin of lower jaw; eyes relatively small, slightly protuberant, pupil oval, transverse, eye diameter slightly equal to snout length (ED/SL 0.75–1.20 in males, 0.83–1.18 in females). Top of the head flat, canthus rostralis rounded and distinct; loreal region weakly concave; nostril round, closer to tip of snout than to eye; interorbital distance greater than internarial distance (IOD/

Figure 2. The *Micryletta hekouensis* alive: from Cuc Phuong NP., Ninh Binh, Vietnam. **A, B.** DTU 316, adult male; **C.** DTU 311, adult male; **D, E.** DTU 317, adult female); from Cat Ba NP, Hai Phong, Vietnam; **F, G.** ZMMU NAP-3580, adult female); from Hekou, Yunnan, China; **H.** KIZ 20210510, holotype, adult male; **I.** KIZ 20210511, paratype, subadult female). Photos by: TV. Nguyen (**A–E**), NA. Poyarkov (**F, G**), and L. Shuo (**H–K**).

IN 1.26–2.16 in males, 1.20–1.71 in females) and upper eyelid width (IOD/UEW 1.45–1.91 in males, 1.30–1.89 in females). Tympanum and supratympanic fold indistinct. Choanae rounded; vomerine teeth absent; opening of vocal sac long cleft; tongue oval, with no notch at posterior tip.

***Forelimbs*:** Forearm length ca. three times shorter than hand length (FLL/HAL 0.43–0.62 in males, 0.40–0.53 in females). Fingers slender with no webbing, rounded in cross-section, no lateral fringes; relative finger lengths: I<II<IV<III; tips of fingers round and not dilated; subarticular tubercles on fingers distinct, rounded and prominent, formula: 1, 1, 2, 2; supernumerary tubercles on palm present and developed; three metacarpal tubercles, inner one rounded and smallest, median one rounded and almost directly in front of oval outer one; two rounded and one elongated prominent supernumerary palmar tubercles on the base of fingers II–IV, respectively; nuptial pad absent.

***Hindlimbs*:** Tibia slightly longer than thigh (FeL/TbL 0.80–0.97 in males, 0.82–1.02), approximately three times longer than wide (TbL/TbW 3.27–5.50 in males, 3.08–5.19 in females); tibiotarsal articulation of adpressed limb reaching eye; foot longer than tibia (TbL/FoL 0.60–0.67 in males, 0.59–0.68 in females). Relative toe lengths: I<II<V<III<IV; tarsal fold absent; tips of toes round and not dilated, slightly wider than those of fingers; webbing between toes absent; subarticular tubercles on toes oval and prominent, formula: 1, 1, 2, 3, 2; dermal ridges present under 2nd to 4th toes but indistinct; inner metatarsal tubercle rounded, prominent, and small; outer metatarsal tubercle absent.

Dorsal skin scattered with small tubercles on dorsum of body, flanks, and hindlimbs, dorsal skin of forelimbs smooth; subtle longitudinal median ridge present on dorsum; dorsolateral fold absent; lateral sides of head smooth; ventral skin of body and limbs smooth.

Colouration in life. Coloration varies greatly, dorsum of body purple brown, blueish grey, or dark brown with two indistinct parallel longitudinal grey stripes on back. Dorsum of forelimbs light yellow, dorsum of hindlimbs the same color as dorsum of body, no bands on dorsum of limbs. Upper lip white. Ventral side of head, body, and limbs greyish brown, purple grey or pinkish brown. Chin region brownish black, males usually have a darker one than females, white marbling patterns on chest and belly, some white spots on chin region and ventral side of limbs. Iris bicolored, with upper third bronze and lower two-thirds brownish black.

Revised diagnosis. Medium-sized within genus *Micryletta* (SVL 20.0–24.1 mm in males, 25.4–29.5 mm in females); areas above canthus rostralis, upper eyelids, areas posterior to eyelids, and dorsum of upper arms golden, other parts of dorsum almost solid black or yellowish grey with brownish black stripes; lateral sides of the head and body black or yellowish grey, a white stripe from lower front of eye along upper lip back to anterior forelimb insertion; ventral side of body and limbs pink brown, chin region in adult males brownish black, small and irregular white marbling patterns on chest and lateral belly; tympanum indistinct; supratympanic fold indistinct; outer metatarsal tubercle absent; webbing between toes absent; tibiotarsal articulation adpressed limb reaching level of eye (data from Liu et al. (2021) and this study).

Comparisons. We summarize the main characters separating *Micryletta hekouensis* from the other twelve species of the genus *Micryletta* in Suppl. material 1. In Vietnam, previously this species was often recorded under the names *M. steinegeri* or *M. inornata*, therefore we focused on comparing the morphological characteristics of *Micryletta hekouensis* with these two species. *Micryletta hekouensis* differs from *M. steinegeri* by having:venter

Table 3. Measurements (in mm) of the specimens of *Micryletta hekouensis* in Vietnam.

Specimen number	Sex	SVL	HW	HL	SL	ED	NSD	END	UEW	IOD	IND	FLL	HAL	fd3D	FeL	TbL	FoL	TbW	td4D	IMTL
DTU 310	**M**	22.2	6.9	6.4	2.5	3.0	1.3	1.6	2.0	2.9	2.3	5.1	11.4	0.7	9.5	9.8	16.3	3.0	0.6	0.9
DTU 316	**M**	24.1	7.2	7.2	2.9	3.0	1.3	1.9	2.0	3.1	2.3	6.0	14.0	0.5	11.6	12.5	20.3	3.3	0.5	0.9
ZMMU NAP-2176	**M**	21.6	6.7	7.2	2.8	2.1	1.8	1.6	1.8	2.7	1.6	5.5	10.8	0.5	8.4	10.5	16.2	2.5	0.5	0.7
ZMMU NAP-3580	**M**	20.0	6.2	6.6	2.7	2.5	1.0	1.4	1.6	3.0	2.2	6.1	9.8	0.5	8.9	9.9	14.9	1.9	0.6	0.6
ZMMU NAP-5572	**M**	23.1	7.9	7.1	3.7	3.0	0.8	2.2	1.3	4.1	1.9	7.4	12.6	0.5	11.6	13.2	19.6	2.4	0.6	0.5
	Min	20.0	6.2	6.4	2.5	2.1	0.8	1.4	1.3	2.7	1.6	5.1	9.8	0.5	8.4	9.8	14.9	1.9	0.5	0.5
	Max	22.2	6.9	6.4	2.5	3.0	1.3	1.6	2.0	2.9	2.3	5.1	11.4	0.7	9.5	9.8	16.3	3.0	0.6	0.9
	Mean	22.2	7.0	6.9	2.9	2.7	1.2	1.7	1.7	3.1	2.1	6.0	11.7	0.5	10.0	11.2	17.5	2.6	0.6	0.7
	SD	1.54	0.63	0.36	0.47	0.40	0.37	0.33	0.33	0.53	0.28	0.85	1.62	0.07	1.49	1.56	2.34	0.52	0.07	0.17
DTU 309	**F (gravid)**	28.9	7.9	7.7	3.2	3.2	1.3	1.9	2.2	3.6	2.5	6.5	14.6	0.7	12.5	13.0	19.8	3.1	0.5	1.6
DTU 311	**F (gravid)**	27.1	7.4	7.6	2.7	3.1	1.3	1.7	1.8	3.4	2.3	5.8	13.3	0.7	11.2	11.7	19.7	3.8	0.8	1.1
DTU 312	**F (gravid)**	29.5	7.6	7.1	2.8	3.3	1.3	1.8	1.8	3.3	2.6	5.3	13.4	0.7	12.4	12.6	19.3	3.6	0.6	1.0
DTU 317	**F (gravid)**	28.7	9.4	8.8	3.3	3.2	1.6	2.1	2.3	3.0	2.5	6.9	15.0	0.6	13.6	13.3	21.7	3.6	0.7	1.0
ZMMU NAP-3352	**F**	25.4	8.9	8.0	3.5	2.9	1.5	2.3	1.7	3.5	2.6	6.5	14.0	0.4	11.5	14.0	20.6	2.7	0.4	0.6
ZMMU NAP-3574	**F**	26.0	9.6	7.9	3.6	3.1	1.4	2.0	2.0	3.9	2.4	7.4	14.0	0.4	12.9	13.0	20.5	2.9	0.4	0.8
ZMMU NAP-5574	**F**	26.8	9.5	8.5	3.3	3.2	0.9	1.7	1.6	4.1	2.4	7.5	15.7	0.6	11.6	13.1	21.5	2.8	0.5	0.4
	Min	25.4	7.4	7.1	2.7	2.9	0.9	1.7	1.6	3.0	2.3	5.3	13.3	0.4	11.2	11.7	19.3	2.7	0.4	0.4
	Max	29.5	9.6	8.8	3.6	3.3	1.6	2.3	2.3	4.1	2.6	7.5	15.7	0.7	13.6	14.0	21.7	3.8	0.8	1.6
	Mean	27.5	8.6	8.0	3.2	3.1	1.3	1.9	1.9	3.6	2.5	6.6	14.3	0.6	12.3	13.0	20.4	3.2	0.6	0.9
	SD	1.55	0.96	0.58	0.34	0.13	0.22	0.23	0.26	0.38	0.11	0.80	0.88	0.14	0.86	0.68	0.92	0.46	0.14	0.37

without dark patterns (vs. with greyish white and brown spots); webbing between toes absent (vs. rudimentary webbing); tibiotarsal articulation adpressed limb reaching level of eye (vs. reaching to tympanum). *Micryletta hekouensis* differs from *M. inornata* sensu stricto by having: lager body size in females (SVL 25.4–29.5 mm vs. 19.5 mm); ventral side of body and limbs pinkish brown or pinkish grey with small and irregular white marbling patterns on chest and lateral belly (vs. reddish grey without mottling, nearly immaculate, or chin, chest, and lateral belly with a few dark marbling patterns).

Distribution. *Micryletta hekouensis* was previously known only from Nanxi Town, Hekou County, Honghe Prefecture, Yunnan Province, China (Liu et al. 2021). We here add further records of this species in Vietnam (Ninh Binh and Hai Phong provinces). The new location in Cat Ba NP is situated ca. 335 airline kilometers southeast of the type locality. Given the geographic proximity and distribution patterns of limestone-associated herpetofauna in Vietnam (see Poyarkov et al. 2021a, 2023), *M. hekouensis* likely occurs in several limestone karst massifs of Northern Vietnam; in particular, records from Quang Ninh, Lang Son and Bac Giang provinces of Northeastern Vietnam, as well as from Hoa Binh, Ha Nam, and Thanh Hoa provinces of Northwestern Vietnam are anticipated.

Natural history notes. Prior to this study, biological data of *Micryletta hekouensis* were very limited; it was only reported from an altitude of 350 m a.s.l. (Liu et al. 2021). The species appears to be closely associated with karstic habitats (Poyarkov et al. 2018; Liu et al. 2021). In Cuc Phuong NP, the frogs were observed from 16:00 to 20:00 h under the dead leaves on the ground. Other species of microhylids recorded syntopically with *Micryletta hekouensis* in Cuc Phuong NP included *Kalophrynus interlineatus* (Blyth, 1855), *Glyphoglossus* cf. *yunnanensis* (Boulenger, 1919), *Kaloula pulchra* Gray, 1831, *Microhyla berdmorei* (Blyth, 1856), *M. butleri* Boulenger, 1900, *M.* cf. *heymonsi* Vogt, 1911, *M. mukhlesuri* Hasan, Islam, Kuramoto, Kurabayashi & Sumida, 2014, *M. pulchra* (Hallowell, 1861), *Micryletta nigromaculata* Poyakov, Nguyen, Duong, Gorin & Yang, 2017, and *Vietnamophryne* cf. *orlovi* Poyarkov, Suwannapoom, Pawangkhanant, Aksornneam, Duong, Korost & Che, 2018. In Cat Ba NP, the frogs were observed from 16:00 to 20:00 h hiding between small pieces of limestone rocks. Other species of microhylids recorded syntopically with *Micryletta hekouensis* in Cat Ba NP included *Kaloula pulchra*, *Microhyla butleri*, *M. fissipes* Boulenger 1884, *M.* cf. *heymonsi*, *M. pulchra*, *Micryletta nigromaculata*. We also recorded gravid females of *M. hekouensis* in June in Cuc Phuong, but other reproduction biology data, such as advertisement call, tadpole morphology, as well as diet of the species remains unknown. It is remarkable that in both localities of *Micryletta hekouensis* in Vietnam, this species was recorded in syntopy with *M. nigromaculata*, another species of the genus *Micryletta* strongly associated with limestone karst formations of northern Vietnam.

Discussion

In this study we re-examined specimens reported by Poyakov et al. (2018, 2021) from karstic habitats in northern Vietnam (Ninh Binh and Hai Phong Provinces) that had previously been assigned to *Micryletta* cf. *inornata* or *M.* cf. *steinegeri* and found that all of these should be assigned to the recently described *Micryletta hekouensis*, representing the first record of this species in Vietnam. The discovery of *Micryletta hekouensis* in this study raises the known number of *Micryletta* species in Vietnam to five, with two species endemic to this country, namely *M. melanops* and *M. nigromaculata*. Consequently, we suggest to remove *Micryletta steinegeri* from the fauna of Vietnam.

Future studies reassessing the taxonomy and distribution of populations of *Micryletta inornata* sensu lato in Vietnam as well as other countries of the Indochinese Region (Laos, Cambodia, Thailand) are required for clarification. Until this work, two species of *Micryletta*, previously confused with *M. inornata* were considered to be endemic to China: *M. menglienica* (until now known only from two locations in Menglian and Mengla counties, Yunnan Province, China) and *Micryletta immaculata* (presently considered to be endemic to Hainan Island, China) (Liu et al. 2021; Poyarkov et al. 2021b; Yang and Poyarkov 2021; Yeung et al. 2023). In our molecular study, we found that populations of *M.* cf. *inornata* reported from Ha Tinh Province, Vietnam and Khammouan Province, Laos, were clustered with *M. immaculata* with minimal genetic distance among them (p = 0.9–2.3%). Meanwhile, populations of *M.* cf. *inornata* from Phrae and Chiang Mai provinces, Thailand and Luangprabang Province, Laos were grouped with *M. menglienica* and were also found to be genetically very closely related to it (p = 0.2–1.5%). Our results suggest that these two species may have a wider distribution than currently recorded. *Micryletta immaculata* is likely distributed in southern China, in northern and central Vietnam, central Laos, while *M. menglienica* likely inhabits southern Yunnan, northern Vietnam, northern Laos, northern Thailand, and possibly also can be found in eastern Myanmar (see Fig. 3). Therefore, re-examination of specimens previously reported as *M. inornata* as well as additional specimens from other locations within the Indochinese Region are required. As noted above, mtDNA markers alone cannot serve as a solid basis for species identification (Solovyeva et al. 2023; Velo-Antón et al. 2023); therefore a thorough examination of phenotypic and multilocus nuclear data is required to obtain a more comprehensive understanding of *Micryletta* diversity and distribution in Indochina.

Micryletta hekouensis is to date known only from two national parks in Northern Vietnam and one region in southern Yunnan; in all three localities these frogs were recorded from a very specific limestone-associated habitat. It is important to notice that karst massifs in Vietnam, as well as in other parts of Southeast Asia, are facing ongoing severe threats from progressing deforestation and

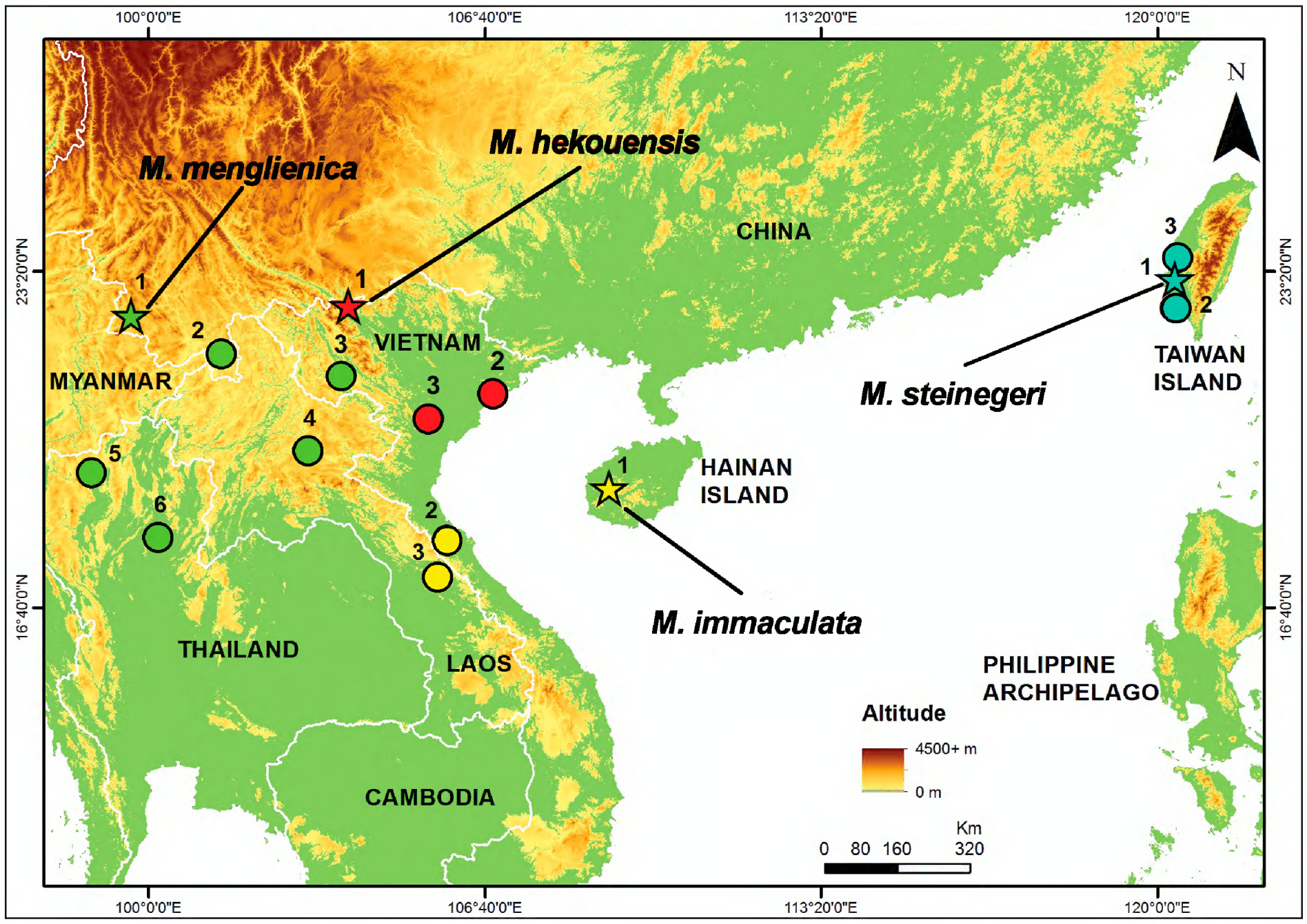

Figure 3. Distribution ranges of the species of the *Micryletta steinegeri* complex. Notes: numbers indicate different localities where the species have been recorded (see Suppl. material 2 for the details of localities)

destruction for cement manufacturing purposes; their continued exploitation for limestone cannot be stopped (Clements et al. 2006; Poyarkov et al. 2021a, 2023). Uncontrolled destruction of limestone massifs may represent the major threatening factor for the species. Although the actual distribution and population status of *Micryletta hekouensis* remains unknown, it is obvious that the species is restricted to isolated highly endangered limestone karst massifs of northern Vietnam and southern Yunnan. Additional surveys in other limestone areas of Northern Vietnam as well as southern Yunnan are essential for elucidating the biology of the species. Our new findings of additional populations of *M. hekouensis* in Vietnam expanded its extent of occurrence (EOO) to 23374 km^2. Given the available information, we suggest *Micryletta hekouensis* to be considered Near Threatened (NT) following IUCN's Red List categories (IUCN 2019).

We also take this opportunity to comment on a recent paper by Pham et al. (2023), who reported on range extension and dietary ecology of *Micryletta nigromaculata* from Son La Province of Northwestern Vietnam. Though the authors claim that the morphological characteristics of the specimens from Son La Province they examined "match well with the diagnosis of *Micryletta nigromaculata*" (Pham et al. 2023: p. 134) this statement is largely misleading, as the photos presented in their paper allow to unambiguously identify this population as *M. menglienica*. For example, in two specimens depicted in Fig. 1 of Pham et al. (2023) white markings are distinctly visible on their upper lips (a feature never observed in *M. nigromaculata*), a light hour-glass shaped pattern on dorsum is absent (always present in *M. nigromaculata*), and body flanks have wide continuous dark bands (vs. black blotches of irregular shape in *M. nigromaculata*) (see Poyarkov et al. 2018). Therefore, even by judging the published photos of both species, the Son La population can hardly be identified as *M. nigromaculata*. Herein, we would like to report that the Son La population described as "*M. nigromaculata*" by Pham et al. (2023) was misidentified and further stress the necessity of accurate comparison of specimens and diagnostic characters with museum vouchers and published information. Furthermore, as *Micryletta* species are often hard to identify by morphological data alone (though not in the case of *M. nigromaculata*), the authors should verify their identification by the means of DNA barcoding, especially in cases when they are not sure about the species identification.

Acknowledgements

We are deeply grateful to Jiangbo Zhao, Jian-Huan Yang (China) and Chung-Wei You (Taiwan) for providing photos of *Micryletta* spp. We also warmly thank Ngoc Quynh

Nguyen (SIFASV, Vietnam) for help in the preparation of the figures. We are grateful to Christopher Oldnall for English proofreading and linguistic help. We thank Günter Gollmann and three anonymous reviewers for kindly reviewing a previous version of the manuscript. NAP and AVT are grateful to Andrei N. Kuznetsov, Svetlana P. Kuznetsova (JVRT STRC), Hoi Dang Nguyen (JVRT STRC), and Leonid P. Korzoun (MSU) for support and organization of fieldwork. This research is supported in part by the Rufford Foundation (Grant No. 39897-1) and the Russian Science Foundation (RSF grant No. 22-14-00037, molecular analyses).

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Supplementary material 1

Morphological comparison of *Micryletta hekouensis* with 13 currently recognized species of the genus *Micryletta*

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Data type: docx

Explanation note: Symbols: (1): SVL (Males, mm); (2) SVL (Females, mm); (3) Iris color; (4) Snout in dorsal view; (5) Foot webbing; (6) Outer metatarsal tubercle; (7) Tibiotarsal articulation of adpressed limb reaching up to; (8) Supratympanic fold; (9) Dorsal skin texture; (10) Dorsal coloration; (11) Dorsal color pattern; (12) Coloration of lateral sides of the head; (13) Coloration of flanks; (14) Ventral coloration.

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Link: https://doi.org/10.3897/herpetozoa.37.e120524.suppl1

Supplementary material 2

List of localities of the *Micryletta steinegeri* group complex appearing on Fig. 3

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Data type: docx

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